measurements also revealed that, just before 16 nm step, the gold particle displaced rightward, perpendicular to the microtubule axis, accompanied with a significant increase in the fluctuation of the gold particle. The rightward asymmetry is consistent with the geometry that the proximal end of the neck linker is located right side of the head. Then the gold particles showed rapid left-forward displacement followed by decrease in the fluctuation, although we could not detect clear sub-step at this temporal resolution. The duration of the highly fluctuating period increased as the ATP concentration was decreased, indicating that the period represents ATP-waiting state. These results provide direct clue to understand how kinesin takes a step forward.

1662-Pos Board B554
Strain-Dependent Regulation of the Kinesin-1's Catalytic Activity as Studied by Disulfide-Crosslinking of the Neck Linker
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Kinesin is a dimeric motor protein that hydrolyzes ATP and moves along microtubules in a hand-over-hand manner. To walk by alternately moving two motor heads, the trailing head should detach from the microtubule prior to the leading head and the detached head should preferentially bind to the forward tubulin-binding site. To explain these mechanisms, we hypothesized that ATP hydrolysis reaction of kinesin motor domain can be regulated depending on the direction of the tension posed to the neck linker: backward strain posed to the neck linker suppresses ATP hydrolysis in the leading head and the forward strain posed to the neck linker suppresses ADP release at the trailing position. To test this hypothesis, we constrained the neck linker in the forward or backward extended conformation using disulfide-crosslinking between cysteine residues on the head and the neck linker, and examined these effects on the microtubule affinity and ADP release kinetics. Single molecule fluorescent observation of the GFP-fused monomeric kinesin showed that when the neck linker was constrained in a backward extended conformation, the dwell time on the microtubule in the presence of saturating ATP was increased by a factor of 15 compared to unconstrained condition. In contrast, stopped-flow measurement showed that when the neck linker was constrained in a forward extended conformation, ADP release rate after microtubule-binding was significantly decreased. These results support the idea that ATP hydrolysis cycle of kinesin's motor domain can be differently regulated depending on the direction of the neck linker extension.

1663-Pos Board B555
A Biosynthetic Approach to Studying Multiple Motor Complexes
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Collective motor dynamics drives important cellular processes ranging from muscle contraction to spindle organization to vesicle trafficking. Although the biomechanical and biochemical properties of individual motors have been widely studied, how motors coordinate their motility when attached to the same cargo is largely unknown. We are developing a biosynthetic approach to generate multi-motor assemblies whose biological properties can be examined in vitro and in cells. To do this, we have assembled a ''toolbox'' of protein components consisting of scaffolds and linkers. We have characterized scaffold proteins of different lengths that allow for specific separation distances between the components. We have characterized four different linker systems that enable constitutive or regulated attachment of individual motors to scaffolds. Thus, our biosynthetic approach can be used to generate multiple motor complexes with absolute control over motor type, separation, and number. The motility properties of these complexes can then be studied in vitro and in live cells to determine the structural and mechanical features that enable kinesin-1 motors to work collectively. This approach is applicable to other biological questions such as the generation of complex signaling networks as well as the assembly of artificial biological systems for engineering applications.

1664-Pos Board B556
Distinct Transport Regimes of Two Elastically Coupled Molecular Motors
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Intracellular transport of cargos is mainly achieved by the cooperative action of molecular motors, which pull the cargos along cytoskeletal filaments. These motors are elastically coupled, which influences the motors' velocity and/or enhances their unbinding from the filament. We show theoretically that interference between two elastically coupled motors leads, in general, to four distinct transport regimes characterized by different effects on the mean velocity and/or the binding time. To gain an intuitive insight in the emergence of these transport regimes, we compare characteristic time scales for the strain force generation. These time scale arguments allow us to predict the transport regimes for different pairs of identical motors. In addition to a weak coupling regime, pairs of kinesin motors and pairs of dynein motors are found to exhibit a strong coupling and an enhanced unbinding regime, whereas pairs myosin motors are predicted to attain a reduced velocity regime. All of the predicted regimes can be explored experimentally by varying the elastic coupling strength.
F. Berger, C. Keller, S. Klumpp, and R. Lipowsky, Phys. Rev. Lett. 108, 208101 (2012)

1665-Pos Board B557
Experimental and Computational Investigations into Cooperative Cargo Transport by Mixtures of Kinesins from Different Families
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Transport of intracellular cargo often involves multiple motor types, either having opposite directionality such as during bidirectional transport of vesicles, or having the same directionality but different speeds. While significant progress has been made in characterizing motors at the single-molecule level, predicting their ensemble behavior is still challenging. To uncover the force-dependent properties of diverse kinesins and to understand how diverse kinesins attached to the same cargo coordinate their movement, we carried out microtubule gliding assays using pairwise mixtures of motors from the kinesin-1, 2, 3, 5 and 7 families. To match their processivities and ensure identical binding to the glass substrate, the motors were fused to the dimerization domain and coil-1 of kinesin-1, and the neck linkers were adjusted to have a uniform length of 14 amino acids. Uniform motor densities were used and microtubule-gliding speeds were measured as the ratio of fast motors varied from 0 to 1. Depending on the motor pair, velocity versus motor fraction curves varied from convex up to nearly linear to convex down. These findings were recapitulated using a coarse-grained computational model of gliding assays. The simulations incorporate force dependent velocities and dissociation rates from the literature along with mechanical interactions between motors bound to the same microtubule. The simulations also suggest that the motor compliance plays a minimal role in the observed gliding speed compared to observations in quantum dots. The gliding assays combined with the modeling allows us to test hypotheses regarding the characteristics of diverse kinesins under predominantly axial load, avoiding the large normal forces inherent in optical tweezer experiments.

1666-Pos Board B558
A Spacer between the Head and Neck Coil of Kinesin-1 Relieves Inhibition Due to Crosslinking of the Heads
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Kinesin-1 is autoinhibited through crosslinking of its two motor domains (heads) by a tail domain (Kaan, et al., Science 333, 883 (2011)), in addition to crosslinking of the heads by attachment to the neck coil (NC). This 'double lockdown' would prevent undocking of the neck linker (NL) and inhibit ADP release. Inhibition by double lockdown was supported the ability of a disulfide crosslink in a S181C mutant to mimic inhibition in the absence of tails. Insertion of a flexible spacer at the junction of the NL and NC could potentially provide enough 'slack' for NL undocking and ADP release. To test this mechanism, a five amino acid spacer was introduced into the S181C mutant (a total of ten additional amino acids in the dimeric construct). At 2 μM MTs, the rate of ADP release was 0.65 s^{-1} and <0.01 s^{-1} for the crosslinked forms with or without the spacer, versus 4.5 s^{-1} when uncrosslinked. Thus ten amino acids of slack per dimer greatly accelerates ADP release, albeit not fully up to the uncrosslinked level. That this spacer is sufficient to largely relieve the inhibition suggests that the inability to undock the NL is the principal cause of the inhibition produced by double lockdown. Studies with longer and shorter spacers will better define the dependence of the ADP release rate on the amount of slack.
The assistance of James Hopkins in cloning and protein isolation is acknowledged. Supported by NIH Grant NS058848.

1667-Pos Board B559
Biochemical Investigations into the Kinesin-2 Chemomechanical Cycle
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Kinesin-2 and dynein motors transport intracellular cargo bidirectionally along both axonemal and cytoplasmic microtubules. These motor activities underlie intraflagellar transport, melanosome dynamics and other vital transport functions in cells, but the mechanism by which the activities of these oppositely-directed motors are coordinated is not well understood. One important question is whether the properties of kinesin-2 motors are specifically tuned for bidirectional transport rather than long-distance plus-ended transport. Consistent with this, kinesin-2 motors were found to detach much more readily than kinesin-1

under hindering loads, and also to rapidly rebind and continue stepping following detachment. To test the hypothesis that kinesin-2 motors are specifically tuned for bidirectional transport, we carried out stopped-flow and steady-state biochemical studies of monomeric and dimeric kinesin-1 and kinesin-2. In solution, kinesin-2 motors were found to have a 30-fold higher affinity for mantADP than unmodified ADP, presumably due to the hydrophobic nature of the mant moiety. Extrapolated ADP off-rates in the presence of microtubules indicated that for unlabeled nucleotide, ADP dissociation is not rate limiting for either kinesin-1 or kinesin-2. Microtubule pelleting experiments indicated that in the ADP state, monomeric kinesin-2 motor domains have a nearly 10-fold higher microtubule affinity than kinesin-1 motor domains. However, this increased microtubule affinity does not translate into enhanced processivity of dimeric kinesin-2 when compared to kinesin-1. This result suggests that kinesin-2 spends a larger fraction of its hydrolysis cycle in the ADP state and thus is more prone to detaching under hindering loads, but following detachment it readily rebinds to the microtubule. This behavior results in a more dynamic competition with dynein that avoids the cargo coming to a complete standstill due to motor stalling.

1668-Pos Board B560

Increased Mechanical Output by a Kinesin Mutant

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Mechanical output by the kinesin motors depends on conformational changes of the motor that transduce and amplify force, coupled to specific nucleotide states. Large conformational changes, detected in previous structural studies, have led to the proposal that distortional changes of the central beta-sheet play an essential role in force transduction by the motor. We now find that mutation of an invariant residue in the central beta-sheet produces unexpected effects on force transduction by the motor, enhancing the velocity of motor movement on microtubules in vitro and the ability of the motor to crosslink and slide microtubules in the oocyte spindle. The mutated residue is in a hairpin loop that can undergo transition into a beta-strand, becoming part of the central beta-sheet. The most severe mutant shows ~2-fold increased motor velocity in microtubule gliding assays and strikingly elongated spindles in vivo. Simulations of spindle assembly indicate that the elongated spindles arise because of the tighter binding by the motor to microtubules and its faster velocity of movement. The mutated residue thus plays a central role in kinesin motor mechanotransduction. Its effects in greatly increasing both ADP release and microtubule binding by the motor indicate a role for the central beta-sheet in coupling the nucleotide- and microtubule-binding sites, and promoting ADP release. We propose that interactions of the mutated residue distort the central beta-sheet, inducing ADP loss and triggering a force-producing stroke. The increased rate of ADP loss by the mutant increases its rate of ATP hydrolysis, accounting for the increased mechanical output by the motor.

1669-Pos Board B561

The Mechanism of the Transition from Diffusive to Directed Movement in Mammalian Cytoplasmic Dynein

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Cytoplasmic dynein is a minus-end-directed molecular motor involving in various cellular functions. Mammalian cytoplasmic dynein has been reported to exhibit unidirectional movements for several micrometers in vivo (Kobayashi, 2009). However, some in vitro studies have reported that single molecules of cytoplasmic dynein/dynactin complex showed biased diffusive movements along microtubules (Ross, 2006; Miura, 2010), and a recent study has demonstrated that an mRNP complex bound by a few dyneins displayed bidirectional diffusive movements (Amrute-Nayak, 2012). To reveal the mechanism of the transition from diffusive to directed movement in cytoplasmic dynein, we designed recombinant dyneins using HEK293 cell expressing system. An artificially dimerized, tail-truncated human cytoplasmic dynein 1 (DHC380) showed unidirectional movement, whereas full-length dyneins purified from the same system displayed biased diffusion towards the microtubule minus end. Furthermore, using several other recombinant dyneins that have different head-to-head distances between two motor heads, we discovered that the dynein with longer head-to-head distance contained larger diffusive component. We also observed that multiple full-length dyneins bound to Qdot moved unidirectionally along microtubules, while single dyneins carrying Qdot exhibited diffusive movements. These observations imply that the directed movement of dynein is influenced strongly by the head-to-head distances and the motor number, possibly leading to the regulation of dynein by these factors in the cell.

1670-Pos Board B562

Src Kinase regulates the Human Kinesin-5, Eg5, by Phosphorylating Tyrosines in the Eg5 Motor Domain

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The human kinesin-5, Eg5, is required to establish and maintain the mitotic spindle. using in silico, in vitro and cell culture methods, we show that Src kinase phosphorylates specific tyrosine residues in Eg5. These residues are located near the nucleotide pocket and the functionally critical L5 loop. Phosphomimetic and non-phosphorylatable Eg5 mutant proteins have diminished ATPase activity and microtubule sliding relative to wild-type Eg5. We also report that phosphomimetic proteins have greatly reduced affinity for the Eg5 inhibitor S-trityl-L-cysteine. This finding suggests that Src phosphorylation of Eg5 may provide cells a non-mutagenesis-dependent strategy to evolve resistance to anti-mitotic Eg5 inhibitors. In this case, combination treatment targeting both SFKs and Eg5 may inhibit mitosis more effectively than anti-Eg5 treatment alone. Ultimately, Src phosphorylation of Eg5 represents a novel regulatory mechanism for a human kinesin, and links the chemical and physical processes that cause mitosis.

1671-Pos Board B563

Effect of Phosphorylation on Motile Properties of the Mitotic Kinesin-5 Cin8

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The mitotic spindle is a microtubule-based bipolar structure which undergoes a well-defined set of morphological changes while mediating the segregation of duplicated chromosomes. It has been established that spindle morphogenesis is governed, in part, by the activity of molecular motors from the kinesin-5 family of bipolar motors with two pairs of catalytic domains located at the opposite sides of the active complex. Kinesin-5 motors are believed to perform their functions by crosslinking and sliding apart antiparallel microtubules originating from opposite spindle poles. Saccharomyces cerevisiaes cells express two kinesin-5 homologues; Cin8 and Kip1 that overlap in essential mitotic functions such as spindle assembly and anaphase spindle elongation.

Previous work from our laboratory indicated that Cin8 is differentially phosphorylated during mid-late anaphase at three cyclin-dependent kinase 1 (Cdk1) specific sites located in its motor domain. Phosphorylation of Cin8 at anaphase, causes its detachment from the spindles, reduces spindle elongation rate and aids in maintaining spindle morphology (Avunie-Masala et al., 2011). To understand how phosphorylation regulates the functions of Cin8, we examined its motile properties by a single-molecule fluorescence motility assay, in which movements of single Cin8-3GFP molecules were observing on fixed microtubules. Since Cin8 was shown to be phosphorylated only during anaphase, we first examined its motile properties in crude extracts of yeast cells arrested in the different points of the cells-cycle. We also compared the motile properties of phosphorylation-deficient and phosphorylation-mimic mutants of Cin8 to the motile properties of the wild type Cin8. We found differences in characteristics of Cin8 motility under the different conditions. Results will be presented.

1672-Pos Board B564

Kinetochore Kinesin CENP-E Tracks the Tips of Dynamic Microtubules via the 'Tethered Motor' Mechanism

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Member of kinesin-7 family CENP-E is a kinetochore-associated plus-end-directed motor, which is important for faithful chromosome segregation in mitosis. CENP-E assists chromosome transport to the spindle midzone, where the microtubule plus ends are located. Here we report that once reaching the dynamic microtubule ends in vitro, CENP-E converts from a lateral transporter into a microtubule tip-tracker, stably associating with the tips of both assembling and disassembling microtubules. We show that the binding between kinetochores and dynamic microtubule ends is destabilized in live cells when CENP-E function is perturbed via an inhibition or RNAi depletion, implying